PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

FACULTAD DE CIENCIAS EXACTAS Y NATURALES

ESCUELA DE CIENCIAS BIOLÓGICAS

ESTRUCTURA GENÉTICA Y ECOLOGÍA DE POBLACIONES DE *Puya hamata* (BROMELIACEAE) EN EL PÁRAMO DEL VOLCÁN CHILES

Disertación previa a la obtención del título de Licenciado en Ciencias Biológicas

GABRIEL SEBASTIÁN RIVADENEIRA GALLEGOS

QUITO, 2016

Certifico que el trabajo de titulación para la Licenciatura en Ciencias Biológicas del candidato Gabriel Sebastián Rivadeneira Gallegos ha sido concluida con conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

Rommel Montúfar, Ph.D.

Director de la Disertación

Quito, 3 de Mayo del 2016

AGRADECIMIENTOS

The work was carried out with permission from the Ecuadorian Ministry of Enviroment (Nº MAE-DPAC-UPN-BD-IC-FLO-2013-003). Thanks to Dr. Paul Ramsay, Dra. Katya Romoleroux and Dr. Hugo Navarrete for their comentaries to this document. A special thanks to Luis López, Rosita Bayas, Patricia Serrano, Ana Troya and Cristian Yerovi for their help during the differents stages of this work.

TABLA DE CONTENIDOS

LISTA DE FIGURAS

LISTA DE TABLAS

1. RESUMEN

La estructura genética de las poblaciones de *Puya hamata*, una planta polinizada por colibríes, semélpara y andina, está definida por dos patrones: dispersión y fuegos de origen antropogénico. Este estudio examina los dos patrones sobre una base genética usando marcadores moleculares (microsatélites). Individuos aislados y seis parches de *P. hamata* fueron escogidos para explorar la estructura genética de las poblaciones (n=74). Veinte infructescencias de individuos aislados y de diferentes parches fueron colectados para explorar la diversidad genética dentro de cada infrutescencia. Los resultados demuestran que cada parche (población) tiende a tener su propia huella genética lo que sugiere que los miembros de un mismo parche descienden de una misma planta madre. Estos parches ponen en potencial desventaja para las poblaciones de *P. hamata* porque el flujo génico está restringido entre estos y como consecuencia esto aumenta la endogamia. Los análisis estadísticos confirman lo propuesto con altos números de alelos tanto en los parches como en las infrutescencias, pero un limitado número de alelos únicos, solo presentes en ciertas poblaciones / infrutescencias. La distribución de *P. hamata* limita el impacto de fijación de alelos entre poblaciones, producido por la deriva génica y reduce el aptitud de las poblaciones, con altos niveles de endogamia. Esta situación debe ser considerada ya que es consecuencia de actividades humanas en los páramos. En un futuro cercano, las poblaciones de *P. hamata* pueden sufrir una erosión a su acervo genético, lo que podría afectar a la evolución de esta especie en el páramo estudiado.

Palabras Clave: Andes; Endogamia; Flujo génico; Colibríes; Microsatélites; Polinización.

2. ABSTRACT

The genetic structure of *Puya hamata*, a hummingbird-pollinated, semelparous, Andean plant species, is defined by two patterns: dispersal and anthropogenic fires. This study examines both patterns into a genetically study using microsatellite markers. Isolated individuals and six patches of *P. hamata* were chosen to explore the genetic structure of populations (n = 74 individuals). 20 infructescences from isolated individuals and from different patches were chosen to explore the genetic diversity inside of each infructescence. The results show that each patch (populations) trends to have its own genetic fingerprint suggesting that individual members of the same patch descend from the same parents. These patches create a potencial handicap for the *P. hamata* populations, because gene flux is restricted to them and as a consequence imbreeding increases. The statistical analyses confirmed the proposed with high levels of allelic numbers in the *P. hamata* populations / infructescences, but a restriction of private alleles to only some populations / infructescences. The actual distribution pattern of *P. hamata* limited the impact of genetic drift by fixing alleles on the populations, and reduce the fitness of populations by high inbreeding levels. Such situations are worthy of attention, because are consequence of human activities in páramo ecosystem. In a near future the *P. hamata* populations could suffer an erosion of their gen pool, that could affect the evolution of this species in this area.

Key Words: Andes; Endogamy; Gene flux; Hummingbirds; Microsatellites; Pollination

3. MANUSCRITO PARA PUBLICACION

REVISTA: Basic and Applied Ecology. Journal of the Ecological Society of Germany, Austria and Switzerland.

TÍTULO: Genetic structure and ecology of *Puya hamata* populations (Bromeliaceae) on the páramo of volcán Chiles.

AUTORES: Rivadeneira Gabriel, Montúfar Rommel

Laboratorio de Genética y Ecología; Escuela de Ciencias Biológicas; Facultad de Ciencias Exactas y Naturales; Pontifica Universidad Católica del Ecuador; Quito-Ecuador.

INTRODUCTION

Located in the neotropic, the páramo is one of the most important biodiversity hotspots on the planet (Madriñán, Cortés, & Richardson 2013)**.** This region is a natural laboratory available for studying the evolution and ecology of plants due to its diversity and endemism, as well as strategies developed by plants to resist the hard high-altitude environment conditions: low oxygen levels, high UV radiation, strong temperature changes and the extreme resource limitation (Carpenter 1978; Willis, Bennett, & Birks 2009). In spite of the diversity of ecological adaptations of páramo species, few studies have been done to explore the link of these strategies to the population genetics.

This research is focused on a representative plant species of the páramo: *Puya hamata* (Bromeliad plant family). This plant species is particularly interesting for the study of populations genetics and ecology due to: (i) this species is a semelparous plant (breeding only once in a lifetime) which produce a long inflorescence (*c.a*. 4 m) with more than 1000 flowers and a flowering period of three months (Miller 1986), (ii*) P. hamata* has a peculiar seed dispersal strategy characterized by an enormous production of small seeds (*c.a.* 600.000 seeds/infructescence) and a limited spatial dispersion restricted mostly near to the parents (Jabaily & Sytsma 2013), (iii) the hummingbirds (Trochilideae family) are the only pollinators of *P. hamata* (Hornung-Leoni & Sosa 2005; Carpenter 1978). Eight species of hummingbirds have been reported sucking on the nectar of *P. hamata* (Woods & Ramsay 2001) and each of them has different strategies depending on the energy-source availability. For instance, *Patagona gigas* applies a forage by trap-linning strategy, flying from one plant to another, whereas *Aglaectis cupripennis* defends its territory against other birds (Feinsinger & Colwell 1978; Woods & Ramsay 2001).

Additionally, the spatial structure and distribution of *P. hamata* populations have a strong link with anthropogenic fire dynamics (Ramsay 2014; García-Meneses & Ramsay 2014). The resistance to fire disturbance (morphological adaptations) gives *P. hamata* an advantage to survive and subsequently germinate its seeds in these disturbed habitats (Mcintyre, Lavorelt, & Tremont 1995). The enormous amount of seeds, restricted dispersion and high germination rates favor individuals of *P. hamata* being able to form a patch in burned areas (García-Meneses 2012).

The goals of the present study are to explore how does population genetics can provide new insights to understand the spatial distribution patterns of *Puya hamata* populations. A population genetics study applying molecular markers (SSRs simple sequence repeats) is going to be used to analyze the following hypothesis: (i) if a *Puya* patch is structured by only a unique parent from which all the individuals descend as proposed by García-Meneses (2012), we would expect low levels of genetic diversity, high endogamy and a strong genetic divergence among populations; and, (ii) if hummingbirds are territorialities and defend each inflorescence avoiding the pollination of other individuals; and particularly inflorescences from the center of the patches should be better protected than those from the border as proposed by García-Meneses (2012), so we expect that central infructescences will show less genetic variability than those from the edge, or even lesser than isolated individuals. We discuss our results based and comparing with the information related to the dispersion and fire impact of páramos plant populations.

The molecular data from leaves and seeds were analyzed at three different levels: (i) Populations Genetic Composition (*PGC*), (ii) Population Arrangement (*PA*), and (iii) Spatial Distribution (*SD*). On the first level (*PGC*) the aim was to identify gene diversity, gene differenciation and gene distance of the populations. The goal of the second level (*PA*) was to cluster the individuals according to genetic similarities. For the last level (*SD*) the aim was to analyze the spatial correlation between genetic data and geographical distances.

METHODS

Study area

The study area was situated on the Páramo of Chiles in the border area between Ecuador and Colombia (Carchi Province; 173248.19m E, 89318.83m N; 18 N). This páramo is constantly used for agriculture and cattle by the residents of the small town called locality of Tufiño (Cuesta, Báez, Muriel, & Salgado 2014). The fire rate is high in the Chiles area, since the Tufiño residents burn the vegetation using fuel; this destructive practice is performed with the aim of getting better grounds for agriculture (Borrelli, Armenteras, Panagos, Modugno, & Schütt 2015). The climate of the study area is characterized by an average annual temperature of 9ºC and a strong topographic gradient (Maya & Bolaños 2011). The local flora are mostly humid *Calamagrostis effusa* grasslands with giant rosettes *Espeletia pycnophylla* (Moscol-Olivera & Cleef 2009). The shrubs *Brachyotum lindenii*, *Diplostephium floribundum* and the herbs *Chaptalia cordata*, *Lupinus pubescens* are common in this páramo (Peyre 2015).

Sampling area

The sampling was composed of two parts that respond to a generational kinship. The first one was about patterns of gene flux among mature individuals, in order to explore genetic structure of *P. hamata* populations. The second one was related to measure the genetic diversity inside of each infructescences from differents patches.

The spatial location of *Puya hamata* populations (mature plants) shows a notorious patchiness distribution. Leaf samples of 74 mature individuals were collected for molecular analysis. We identified three paterns: (i) isolated individuals, (ii) individuals gathered in small to medium groups (5 to 10 individuals), and (iii) individuals organized into big groups (>20 individuals). From this clasification we choose the following samples: 15 isolated plants located between patches, hereafter named as P (P1-P15); three small populations (SG1, SG2, SG3), from which we sampled 11, 3 and 11 individuals, respectively; and three big populations were selected (BG1, BG2, BG3), from which we sampled 10, 12 and 12 individuals, respectively. The isolated individuals (P) were grouped in a unique set for statistic analysis (**Fig 1**).

Seeds samples (4 to 10) from 20 infructescenses were collected aleatory to evaluate the effect of pollination on the genetic diversity among the seeds of the same infructescence. *Puya hamata* individuals has only one infructescence per individual. These seeds populations came from: (i) six isolated individuals, (ii) four individuals that belong to small groups, and (iii) ten individuals from a big group. The latter category was split out in two levels: 5 individuals from the edge of the group and 5 individuals from the center of the group. Hereafter, the seeds from isolated individuals will be refered as IS, the seeds from small groups as SS, the seeds from the edge of the big groups as EB and the seeds from the center of the big group as CB. Ten infructescences belong to the big group (5 CB and 5 EB) and the other ten are scattered in the study area (**Fig 2**).

The mature individuals and the seeds were collected during two sampling periods. The first one was on August 2013 and the second period was on February 2014. For mature individuals samplings a small piece of leaf tissue was collected to molecular analysis. The samples were stored in plastic bags with silica gel.

DNA Extraction, primer screening and genotyping

DNA extraction from the leaf tissue was carried out according to the Doyle and Doyle 1987 method modified. DNA extraction from the seeds was done using the Wizard Genomic DNA Purification Kit Promega following the manufacturer's instruction. After extractions, the DNA isolated of each sample was quantified with a Nanodrop spectrophotometer (Thermo Scientific). A set of 20 microsatellite markers (SRR – simple sequence repeats) related to the genus *Puya* were chosen to be transferred to *P. hamata*. PCR amplifications were performed in a final volumen of 20µl as follows: 5µl of DNA template (2 ng/µl), 1.5mmol MgCl2, 5 µL 5X, DNTPs, 0.35 µL of each primmer, 100 µM dNTPs mix, 0.5 U GoTaq polymerase (Promega). Thermal cycling conditions consisted of an initial denaturation step for 6 min at 94 °C followed by 30 cycles of 30 s at 94°C, 90 s at 65ºC-54°C, and 90s at 72°C. The annealing temperature was reduced by 1ºC per cycle, during the first 11 cycles and left constant. A final 10 minutes extension step at 72°C was added. The PCR products were separated by eletrophoresis in 6% denaturing polyacrylamide gels and by silver nitrate staining. The genetic analyses were performed at the Pontifical Catholic University of Ecuador.

Statistical Analysis

In order to detect the genetic structure of populations (PGC), the inbreeding values (F_{is}) and F_{st} values were computed using Arlequin 3.1 (Schneider, Roessli & Excoffier 2000); the average number of alleles (*A*), the number of private alleles (*PA*), expected heterozygocity (H_e) and oberved heterozygocity (H_o) were obtained through the GeneAleX software (Peakall & Smouse 2012). The populations arrangement (*PA*) was performed with the GeneAleX software using a principal coordinates analysis

(PCoA) with the genetic distance of Nei (Peakall & Smouse 2012) using data derived from leaves and seeds. Additionally, a Bayesian analsyis was performed to determine the populations arrangement (*PA*) among individuals with the Structure software (Evanno, Regnaut, & Goudet 2005) using five independent Markov chain Monte Carlo (MCMC) runs were performed using 10^4 burn-in generations followed by 10^5 sampling generations. For the spatial distribution analysis (*SD*), a distance geographical matrix was built with the geographical coordinates of all individuals using the ArcGis software (Elhorst 2010). The geographical and the genetical matrix were correlated using the Mantel test as implemented by the GenAlex software with 10000 permutations (Diniz-Filho et al., 2013).

RESULTS

Molecular markers (SSRs)

Molecular markers (SSRs) from two close related species were transferred to *Puya hamata*: five SRRs from *Ananas comosus* (Acom 64.22, Acom 78.4, Acom 101.1, Acom 117.15 and Acom 119.1) and one SRRs from *Aechmea caudata* (Ac40) (Goetze et al., 2013; Wöhrmann & Weising 2011). The loci Acom 64.22 was monomorphic, meanwhile the other locus were polimorphic with (3 to 13) alelles. Individuals or seeds with a negative amplification (minimun four positive loci) were deleted from this study. The total number of genotyping samples were 261 individuals: 74 from leaves and 187 from seeds. Five microsatellites markers were used on all statistical analysis.

Populations genetic composition (PGC)

For leave samples, each mature group had in average 11.55 individuals with a maximun of 15 and a minimum of three. The three small populations (SG) presented high values of allele diversity with a maximun of 62 alleles for SG3 among 11 individuals. A similar pattern was reported about big populations (BG) with a maximun of 66 into 12 indivuals (BG2). However, isolated individuals (P) reported the highest allele diversity with 82 alleles into 15 individuals. Private alleles were only reported for isolated individuals (2), and two big groups (LG1=1, LG2=1). The highest values of genetic diversity (H*e*) were found in isolated groups of individuals (0.47) and LG3 (0.48). Endogamy (F_{is}) was negative for isolated individuals (-0.33) and SG3 (-0.44) but high for SG1 (0.21), SG2 (0.6) and LG3 (0.33) (**Table 1**).

For seed samples, the mean sample size per infructescense is 9.35 seeds (maximum = 10; minimum = 4). The average number of alelles in the 20 infructescense was (46.15 ± 10.51). The isolated group IS (6 infructescense) showed an average of 56.83 alleles; individuals from small groups SS (4 infructescense) reported 55.75 alleles; individuals from the edge of the big group EB (5 infructescense) had 50.2 alleles; and individuals from the center of the big group CB (5 infructescense) showed 49.6 alleles. The five private alleles reported were distributed only to the isolated infructescence: IS3 (1), IS5 (3) and IS8 (1). The genetic diversity (H*e*) followed a similar pattern as reported by allelic diversity isolated individuals ($\overline{X}$ = 0.46), small populations ($\overline{X}$ = 0.48), infructescence from the edge of the big patches ($\overline{X}$ = 0.41) and infructescence from the center of the big patches ($\overline{X}$= 0.32). The F_{is} statistics in average was negative for almost all individuals (17 individuals, -0.27 ± 0.25) (**Table 2**).

The genetic distance among mature individuals was depicted by a PCoA analysis. This test showed three groups in the ordination; hereafter the groups will be named by capitals A, B and C. Group A is composed of individuals from population BG2 (12) plus one from BG1 and one from BG3. Group B is composed by individuals from populations SG1 (6), SG2 (2), SG3 (9), LG1 (7), LG3 (3) and P (5). Groups C is composed by individuals from populations SG1 (5), SG2 (1), SG3 (2), LG1 (2), LG3 (7) and P (5). 59 individuals were split up between the groups B and C; meanwhile the isolated individuals (P) were present in groups B and C, and only four individuals were placed on an intermediate space between the three groups. In such intermediate space there were also one individual from BG1 and one from BG3 (**Fig 3A**).

The PCoA analysis using the genetic pattern of seeds coming from 20 infructescences (table 2) showed four groups: (i) group W is composed exclusively by 4 CB infructescences, (ii) group X is composed exclusively by all the 5 EB infructescences and IS3, (iii) group Y is composed by 3 IS infructescences and one CB infructescence, and (iv) group Z is composed exclusively by the 4 SS infructescences. The isolated infructescences which did not belong to any group (WXYZ) were located in the intermediate space among these four groups (IS7 and IS10) (**Fig 4A**).

Population arrangement (PA)

The Bayesianan analysis (Structure) showed similar patterns as reported in the PCoA analysis for mature individuals. Based on the ΔK statistic derived from Structure analysis the best K (clusters number) was 2 (**Fig 3B**). The K1 is composed by 100% of the individuals from BG2, 20% from BG1, 16.67% from BG3 and 6.67% from P. K2 consists of 100% from all the three SG individuals, 93.33% from P, 83.33% from BG3 and 80% from BG1. X-axis of PCoA explains partially the separation of individuals: to the right side stays cluster K1 and on the left side stays cluster K2 (**Fig 3A**). The composition of K2 compared to the PCoA clusters indicates that both groups (B and C) form a unique configuration.

Regarding to the infructescences data, the best cluster number was K = 4, based on the ΔK statistic (**Fig 4B**). Cluster K1 is composed by four infructescences CB, 2 IS and 1 EB. Cluster K2 is composed only by isolated infructescences and share some individuals coming from IS7 with K1. Cluster K3 is composed by infructescences from EB populations and share the infructescences EB1 and IS3 with K1. Group K4 is the only one that does not share any individuals of these infructescences with the other clusters and is composed by all SS and CB1 (**Fig 4A**).

Spatial distribution (SD)

The Mantel test showed a weak but statistically significant correlation (R^2 = 0.0601; $p < 0.0000$**) between genetic distance of mature individuals and the geographical distance. In order to evaluate the impact of BG2 on spatial correlation of samples, a Mantel test was performed with mature individuals, but excluding BG2 individuals; the results showed a reduction of correlation index (R^2 = 0.0297) and it remained statically significant. As reported in the former results, the Mantel test showed a weak but statistically significant correlation (R^2 = 0.0311; $p < 0.0000$**) between genetic distance of infructescences and the geographical distance.

DISCUSSION

Transfer of Microsatellites to *Puya hamata*

Molecular markers have been widely used in the Bromeliaceae family (Hmeljevski et al., 2013) (Zanella et al., 2012), for instance, the genus *Ananas* has been studied extensively by applying molecular markers as microsatellites mainly for breeding issues (Rodríguez, Grajal-Martín, Isidrón, Petit, & Hormaza 2013). However, for Puya genus no microsatellite have been isolated. An important result of this research was the successful transfer of microsatellites markers, isolated originally from genera *Ananas* and *Aechmea* to *Puya*.

The DNA isolated from *Puya* leaves had a low-quality level; this pattern has been formely reported from other genera coming from this family (Schulte et al., 2010). This feature could be explained by: (i) interference in the DNA extracction of secundary compounds that the plant produced to resist the extreme environmental conditions (cold/high UV radiation (Porebski, Bailey, & Baum 1997), and (ii) the leaves of these sexually mature individuals might have been too old and the DNA content was minimal (Murray 1980). In comparison with leaves, the DNA isolated from *Puya* seeds had a better quality in spite of its small size < 2mm (Vadillo, Suni, & Cano 2004).

Mature individuals (leaf samples)

Population Genetic Composition (PGC)

In non human affected páramo, the *P. hamata* individuals are scattered dispersed on the landscape. However, in our study area the antropogenic fires have changed the spatial distribution of *Puya* (García-Meneses & Ramsay 2012). The antropogenic fires remove the native dense tussock vegetation and consequently, create new abiotic conditions (soil, temperature, humidity, organic matter) that favour the germination of *Puya* seeds (Lægaard 1992). Under these abiotic conditions the spatial distribution of *Puya* changes dramatically from scattered to a clustered pattern. Additionally, the seed dispersal of *Puya* is inefficient to move seeds far away from the parent, favoring the formation of patches derived from a unique parent (Benzing 2000). The results of our study confirm the short distance distribution patterns, all the individuals that belong to small or big patches (SG or BG) trend to show a similar genetic fingerprint, which could reduce the gene pool and potentially increase recessive diseases (Charlesworth, Charlesworth, Willis, & Willis 2009). Meanwhile, isolated individuals showed a different genetic fingerprint with higher values of genetic diversity (A, *PA* and H*e*) than individuals coming from the patches (**Table 1**).

Four patches of *P. hamata*, including isolated individuals, showed an excess of heterozygotes (negative values for inbreeding coefficients -F_{is}), it means that these populations are under a heterosis effect (Schnable & Springer 2013). This effect constitutes a potential harm to populations because heterosis does not contribute to increasing the gene pool (Allendorf 1986).

Population arrangement (PA) and Spatial Distribution (SD)

The overall pattern showed on the PCoA is partially explained by an unexpected variable, the altitude. The PcoA based on genetic distance showed three clusters (A, B, C; figure 3). Individuals from B and C cluster are spatially isolated but placed at the same altitude (3600 masl); meanwhile, individuals from cluster A are exclusively placed in the middle of B and C, but in a higher altitude (4000 masl). This pattern was also reported by the Bayesian analysis where the results suggest that altitude is an important force outlining the genetic structure of *P. hamata* population. For instance, individuals from cluster K1 are located in a higher position that the remaining of the *Puya* individuals (approx. 400 meters higher). Spatial distribution of individuals on the landscape is not explained by the physical barriers; however incidental evidence like altitude could explains the genetic flux among population. This result agrees with the ecology behavior of hummingbirds. It has been reported that hummingbirds avoid changing their feeding altitudinal gradient (Salinas 2007); hummingbirds stay at the same altitude moving pollen at the same altitude level during foraging (Carpenter 1978). Additionally to altitude, the distribution of *Puya* individuals was also influenced by the deficient dispersal of seeds.

Seeds

Population Genetic Composition (PGC)

The metabolic rate of hummingbirds needs enormous amounts of energy, so they must feed regularly (Toloza-Moreno, León-Camargo, & Rosero-Lasprilla 2015). Usually, the hummingbird-pollinated plants are rich in sucrose, but *P. hamata* has a lower concentration of sucrose in its nectar (Woods & Ramsay 2001). This irregular food-resource, depends solely upon the time that takes to the plant to reach its maturity state and which is around 30 years (Hornung-Leoni & Sosa 2005). When a *P. hamata* has reached a mature state, automatically become an atractive food-source for hummingbirds. This attractive phenological behavoir could explain why 17 of 20 infructescense showed high values of heterosis (Holsinger & Weir 2009). The high needs of energy impulse hummingbirds to move from infructescense to infructescense moving pollen and increasing alleles movements. The 20 infructescense studied (with ten seeds each) showed a similar average number of alleles (61.77), suggesting that all the individuals were intensively pollinated by hungry hummingbirds that moved constantly among inflorescenses. However, the individuals which reported private alleles came from three of six isolated individuals (**Table 1**). This result could be explained by the feeding strategy of the hummingbird *Aglaectis cupripennis*, an identified pollinator of *P. hamata* on the study area (Woods & Ramsay 2001). This hummingbird defends all inflorescence in its territory (patches) favoring active genetic flux among *Puya* inflorescences under its custody; meanwhile isolated individuals of *Puya* are potentially pollinated by other hummingbirds (Feinsinger & Colwell 1978).

We propose the following scenario to explain the genetic structure of *Puya* populations in our study. We hypothesize that a solitary *Puya* plant, with fire morphological resistance, can survive on a burned area. As it has already been explained this individual would be an atractive source of nectar for hummingbirds, and after the fructification period all the seeds will drop close to the parent. The high germinative rate reported for *Puya* (Jabaily & Sytsma 2013) increases the survival rate for seedlings near to the parent. Some seedlings will grow until they get to a mature state and form a patch (small or big). All the *Puya* individuals of each patch come from a unique parent, so all individuals are genetically related. If *Aglaectis cupripennis* starts to defend a single patch, it will provoke minimal chances of external pollinitation of others hummingbirds. Consequently, for the next pollinization events, all individuals from a same patch will receive only pollen of individuals that are closely related (same patch). The *P. hamata* individuals from the center of the patch are better defended than the ones on the edge, so the chances of been pollinated by external burglar hummingbirds are lower. The fact that the average number of alleles is higher in seeds obtained from infructescence of edge than those from the center constitute an evidence of the defense strategy inside each of the patches as proposed by Garcìa-Meneses & Ramsay (2012). The impact of this behavior is evidenced in the genetic gradient (allele number and genetic diversity) detected in the infructescence analysis; where genetic diversity increase linearly from infructescence of the center of the patch toward the isolated individuals.

As reported previously for spatial distribution of mature individuals, the altitude also outlines the genetic patterns among seed and infructescence of *P. hamata*. Such information confirms that hummingbird pollen dispersal is strongly constrained by altitude and consequently, a major factor modeling the genetic dynamics of *P. hamata* populations.

In conclusion this study hypothesize that: (i) the restricted seed dispersal, (ii) the fire history and (iii) the foraging behavior of *Aglaectis cupripennis* define the genetic composition of *Puya hamata* in the study area. Each *P. hamata* patch behaves like a unique genetic entity with a similar genetic structure, outlined by fire, altitude and dispersal dynamics. We also conclude that, the isolated individuals (P and IS) are the genetics reservoirs of the populations since they are spatially placed among patches, allowing dispersers to eventually move pollen from this isolated individual to patches, therefore increasing the genetic pool of *P. hamata* populations. For small populations like those of *P. hamata* the moving of new alleles from isolated individuals could have an important effect on maintaining its genetic diversity. The actual distribution pattern of *P. hamata* could represent a handicap to the long-term evolution of this species: (i) high heterosis suggests a limited impact of genetic drift by fixing alleles on the populations, and (ii) high inbreeding values reduce the fitness of populations. Into an evolutionary scale, genetic drift and imbreding could produce an erosion of the gen pool of *Puya hamata* populations.

REFERENCES

Allendorf, F. W. (1986). Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology*, 5, 181–190.

Benzing, D. H. (2000). *Bromeliaceae: Profile of an adaptive radiation*. Cambridge: Cambridge University Press.

Borrelli, P., Armenteras, D., Panagos, P., Modugno, S., & Schätt, B. (2015). The implications of fire management in the andean paramo: A preliminary assessment using satellite remote sensing. *Remote Sensing*, 7, 11061–11082.

Carpenter, F.L. (1978). A spectrum of nectar-eater communities. *American Zoologist*, 18, 809-819.

Charlesworth, D., Charlesworth, D., Willis, J. H., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews. Genetics*, 10, 783–796.

Cuesta, F., Báez, S., Muriel, P., & Salgado, S. (2014). La vegetación de los páramos del Ecuador. *Avances En Investigación Para La Conservación de Los Páramos Andinos*, 143.

Davies, T. J., Savolainen, V., Chase, M. W., Moat, J., & Barraclough, T. G. (2004). Environmental energy and evolutionary rates in flowering plants. *Proceedings of the Royal Society B: Biological Sciences*, 271, 2195–2200.

Diniz-Filho, J. A. F., Soares, T. N., Lima, J. S., Dobrovolski, R., Landeiro, V. L., Telles, M. P. de C., Bini, L. M. (2013). Mantel test in population genetics. *Genetics and Molecular Biology*, *36*, 475–485.

Elhorst, J. P. (2010). Handbook of Applied Spatial Analysis. *Methods*, 377–407.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14, 2611–2620.

Feinsinger, P., & Colwell, R. (1978). Community organization among neotropical nectar feeding birds. *American Zoologist*, 18, 779–795.

García-Meneses, P. M. ., & Ramsay, P. M. . (2014). *Puya hamata* demography as an indicator of recent fire history in the páramo of El Ángel and Volcán Chiles, Ecuador-Colombia. *Caldasia*, *36*, 53–69.

García-Meneses, P. M., & Ramsay, P. M. (2012). Pollinator response to within-patch spatial context determines reproductive output of a giant rosette plant. *Basic and*

Applied Ecology, 6, 516–523.

Goetze, M., Batista, R., Lapa, G., Moura, L., Souza, D., Bered, F., & Palma-silva, C. (2013). Development of microsatellite markers for genetic diversity analysis of *Aechmea caudata* (Bromeliaceae) and cross-species amplification in other bromeliads. *Biochemical Systematics and Ecology*, 48, 194–198.

Hmeljevski, K. V., Ciampi, M. B., Baldauf, C., Sedrez, M., Reis, D., & Forzza, R. C. (2013). Development of SSR Markers for *Encholirium horridum* (Bromeliaceae) and Transferability to Other Pitcairnioideae. *Applications in Plant Sciences*, 4, 1245–1250.

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting F(ST). *Nature Reviews. Genetics*, 10, 639–650.

Hornung-Leoni, C., & Sosa, V. (2005). Morphological variation in *Puya* (Bromeliaceae): An allometric study. *Plant Systematics and Evolution*, 256, 35–53.

Jabaily, R. S., & Sytsma, K. J. (2013). Historical biogeography and life-history evolution of Andean *Puya* (Bromeliaceae). *Botanical Journal of the Linnean Society*, 171, 201–224.

Lægaard, S. (1992). Influence of fire in the grass páramo vegetation of Ecuador. In H. Balslev, & J. L. Luteyn (Eds.), *Páramo: An Andean ecosystem under human influence*. London: Academic Press.

Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, 4, 1–7.

Maya-Pantoja, J., & Bolaños-López, A. (2011). Conservación y uso sostenible en la zona de amortiguamiento del páramo de Chiles, departamento de Nariño. *Revista de Investigación Agraria y Ambiental*, 2, 75–82.

Mcintyre, S., Lavorelt, S., & Tremont, R. M. (1995). Plant life-history attributes : their relationship to in herbaceous disturbance response vegetation. *Journal of Ecology*, 83, 31–44.

Miller, G. A. (1986). Oecologia of *Puya* (Bromeliaceae) in the Ecuadorian Andes, 155–160.

Moscol-Olivera, M. & Cleef, M. (2009). A phytosociological study of the páramo along two altitudinal transects. *Phytocoenologia Band*, 39, 79 - 107.

Murray,M., & Thomson, W. (1980) Rapid isolation of high molecular weight plant DNA. *Carnegie Institute, Departamente of Plant Biology*, 8, 19.

Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537–2539.

Peyre, G. (2015). Plant diversityand vegetation of the Andean Páramo. University of Barcelona, Faculty of Biology, PhD Program Biodiversity.

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, 15, 8–15.

Ramsay, P. M. (2014). Giant rosette plant morphology as an indicator of recent fire history in Andean páramo grasslands. *Ecological Indicators*, 45, 37–44.

Rodríguez, D., Grajal-Martín, M. J., Isidrón, M., Petit, S., & Hormaza, J. I. (2013). Polymorphic microsatellite markers in pineapple (*Ananas comosus* (L.) Merrill). *Scientia Horticulturae*, 156, 127–130.

Salinas Letty, C. A. (2007). El néctar de especies de puya como recurso para picaflores altoandinos de ancash, perú. *Revista Peruana de Biología*, 14, 129–134.

Schnable, P. S., & Springer, N. M. (2013). Progress Toward Understanding Heterosis in Crop Plants. *Annual Review of Plant Biology*, 64, 71–88.

Schneider, S., Roessli, D., & Excoffier, L. (2000). Arlequin, Version 2.0: A software for population genetics data analysis. *A Software for Population Genetics Data*, 111.

Schulte, K., Silvestro, D., Kiehlmann, E., Vesely, S., Novoa, P., & Zizka, G. (2010). Detection of recent hybridization between sympatric Chilean Puya species (Bromeliaceae) using AFLP markers and reconstruction of complex relationships. *Molecular Phylogenetics and Evolution*, 57, 1105–1119.

Toloza-Moreno, D. L., León-Camargo, D. A., & Rosero-Lasprilla, L. (2015). El ciclo anual de una comunidad de colibríes (Trochilidae) en bosques altoandinos intactos y paramizados en la Cordillera Oriental de Colombia. *Ornitologia Colombiana*, 14, 28–47.

Vadillo, G., Suni, M., & Cano, A. (2004). Viabilidad y germinación de semillas de *Puya raimondii* Harms (Bromeliaceae). *Revista Peruana de Biología*, 11, 71–78.

Willis, K. J., Bennett, K. D., & Birks, H. J. B. (2009). Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. *Journal of Biogeography*, 9, 1630–1644.

Wöhrmann, T., & Weising, K. (2011). In silico mining for simple sequence repeat loci in a pineapple expressed sequence tag database and cross-species amplification of EST-

SSR markers across Bromeliaceae. *Theoretical and Applied Genetics*, 4, 635–647.

Woods, S., & Ramsay, P. M. (2001). Variability in nectar supply: implications for high-altitude hummingbirds. In P. M. Ramsay (Ed.), *The ecology of Volcán Chiles: High altitude ecosystem on the Ecuador-Colombia border*, 209–217. Plymouth, Uk: Pebble and Shell.

Zanella, C. M., Janke, A., Paggi, G. M., Goetze, M., Reis, M. S., & Bered, F. (2012). Microsatellites in the endangered species *Dyckia distachya* (Bromeliaceae) and cross-amplification in other bromeliads. *International Journal of Molecular Sciences*, 12, 15859–15866.

4. NORMAS PARA PUBLICACION

Instrucciones para autores

Type manuscripts double-spaced throughout and with wide margins. Number pages and lines consecutively. Type genus and species names in italics. Manuscripts should be as short as possible, but no longer than 4,500 words (including the complete text with references and legends, excluding tables and figures) and with titles of less than 15 words and abstracts of less than 300 words.

(a) ***Title*****.** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

(b) ***Summary.*** A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. The English abstract should be followed by a German summary "Zusammenfassung"). Authors who are German native speakers are requested to prepare the Zusammenfassung themselves. If no German native speaker is among the authors, the German summary will be provided by the Editors.

(c) ***Keywords*****.** Immediately after the abstract, provide a maximum of 10 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

(d) ***Acknowledgements.*** Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

(e) ***Introduction.*** State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

(f) ***Material and methods.*** Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

(g) ***Results*****.** Results should be clear and concise.

(f) ***Discussion*****.** This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is sometimes appropriate. Avoid extensive citations and discussion of published literature.

(g) ***Conclusions.*** The main conclusions of the study may be presented in a short conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

(h) ***Figures*****.** Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used. Parenthesized upper case letters should be used to identify sub-figures, both in the figure itself and the figure caption.

(i) ***Tables*****.** Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

(j) ***References.*** *Text:* Citations in the text should follow the referencing style used by the American Psychological Association. You are referred to the Publication Manual of the American Psychological Association, Sixth Edition, ISBN 978-1-4338-0561-5.

5. FIGURES

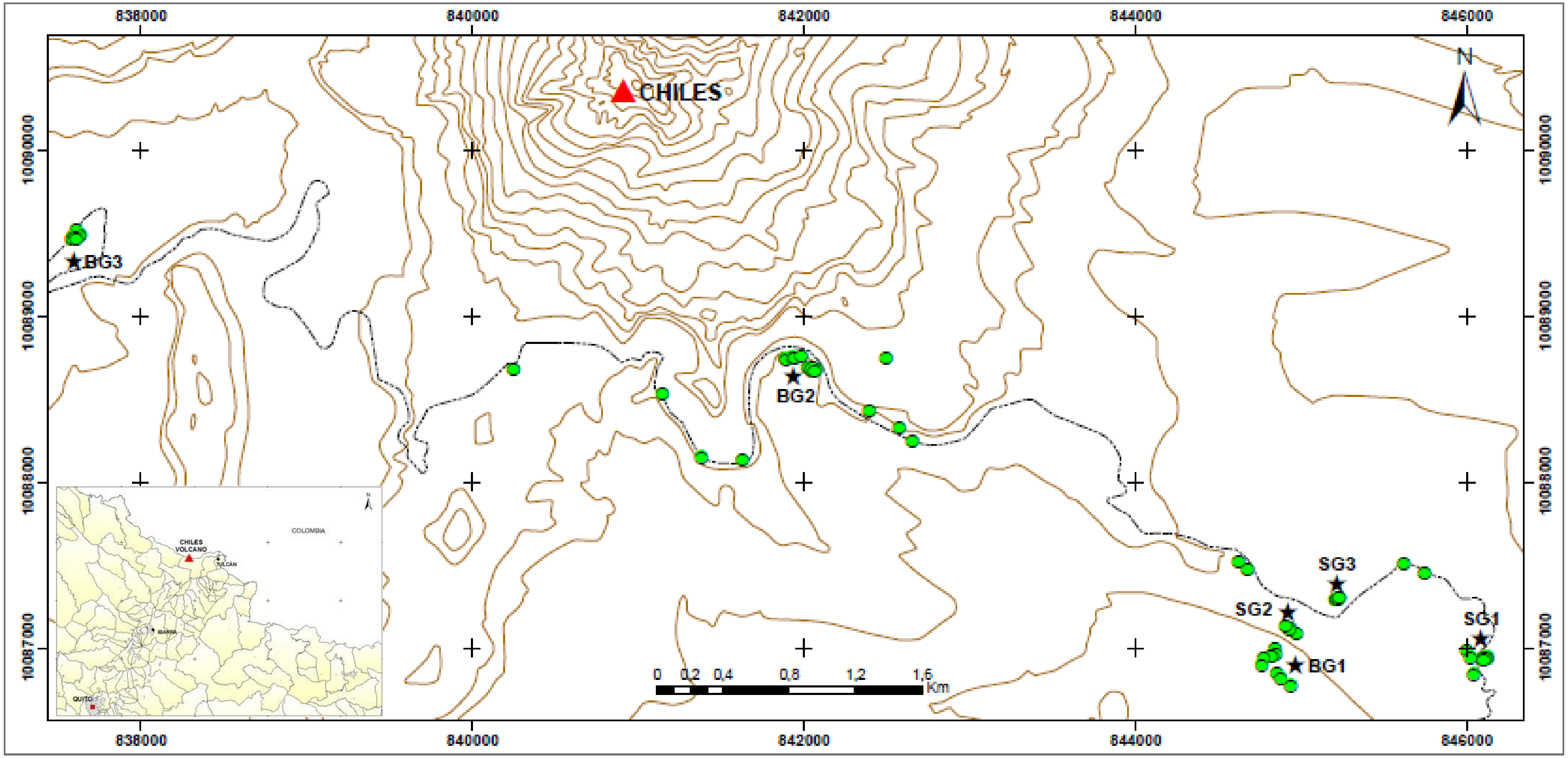

Figure 1. Location of the 74 sexually mature *Puya hamata* individuals. The circles correspond to isolated individuals (P) and the stars to the two different categories of patches (small groups SG / large groups LG). There are 15 isolated individuals (P); three small groups: SG1, SG2, SG3; and there are three large groups: LG1, LG2 and LG3. On this map the contours are clearly visible, the higher point is the Chiles volcano.

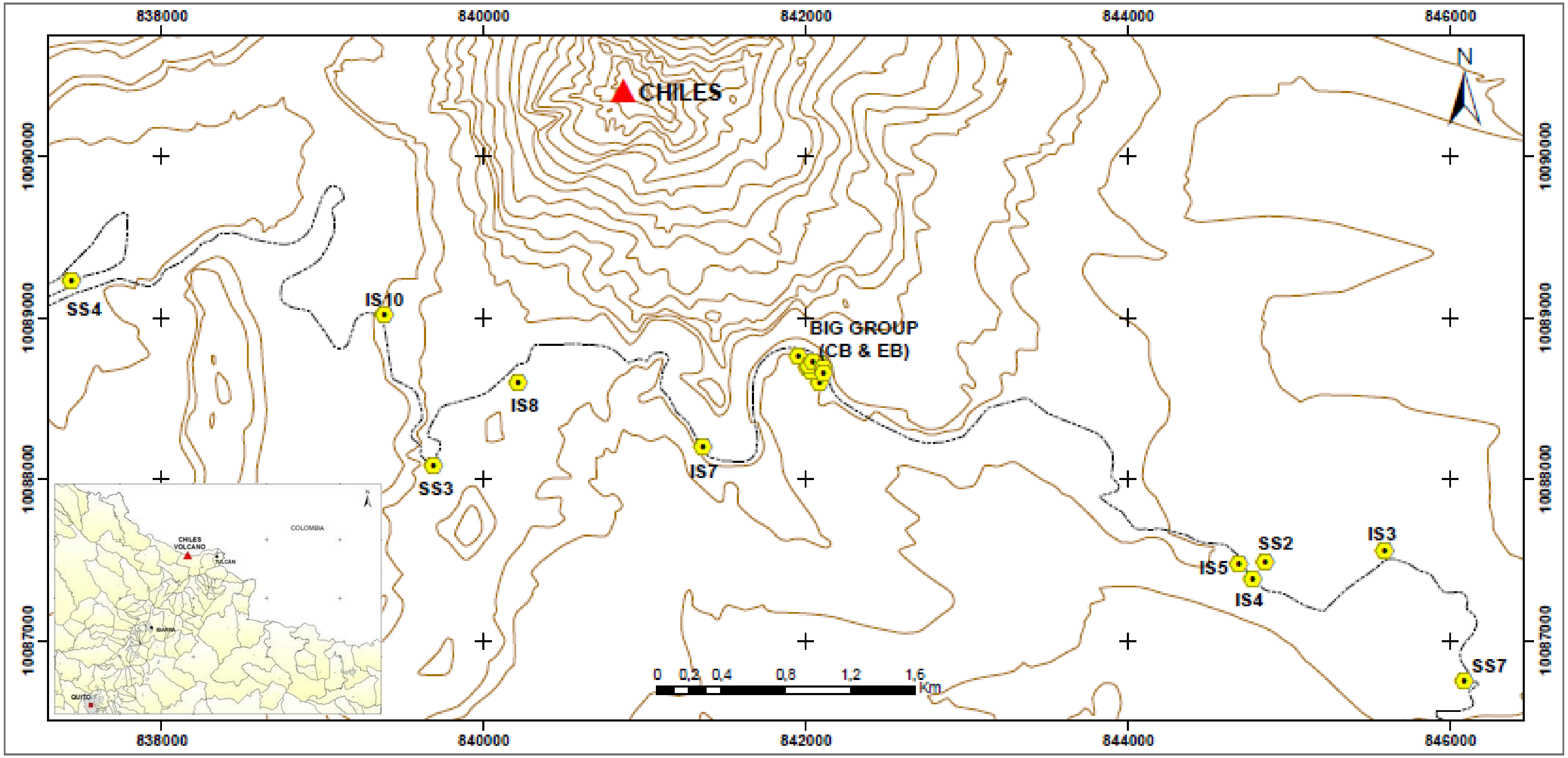

Figure 2. Location of the 20 infructescences of *Puya hamata* seeds. The hexagons correspond to isolated infructescences (IS), Infructescences of small groups (SS) and infructescences of the center or the edge of a big group (EB and CB). There are six isolated infructescences: IS3, IS4, IS5, IS7, IS8 and IS10; four infructescences of small groups SS2, SS3, SS4 and SS7; and ten infructescences of the big group CB1, CB2, CB3, CB4, CB5, EB1, EB2, EB3, EB4 and EB5. On this map the contours are clearly visible, the higher point is the Chiles volcano.

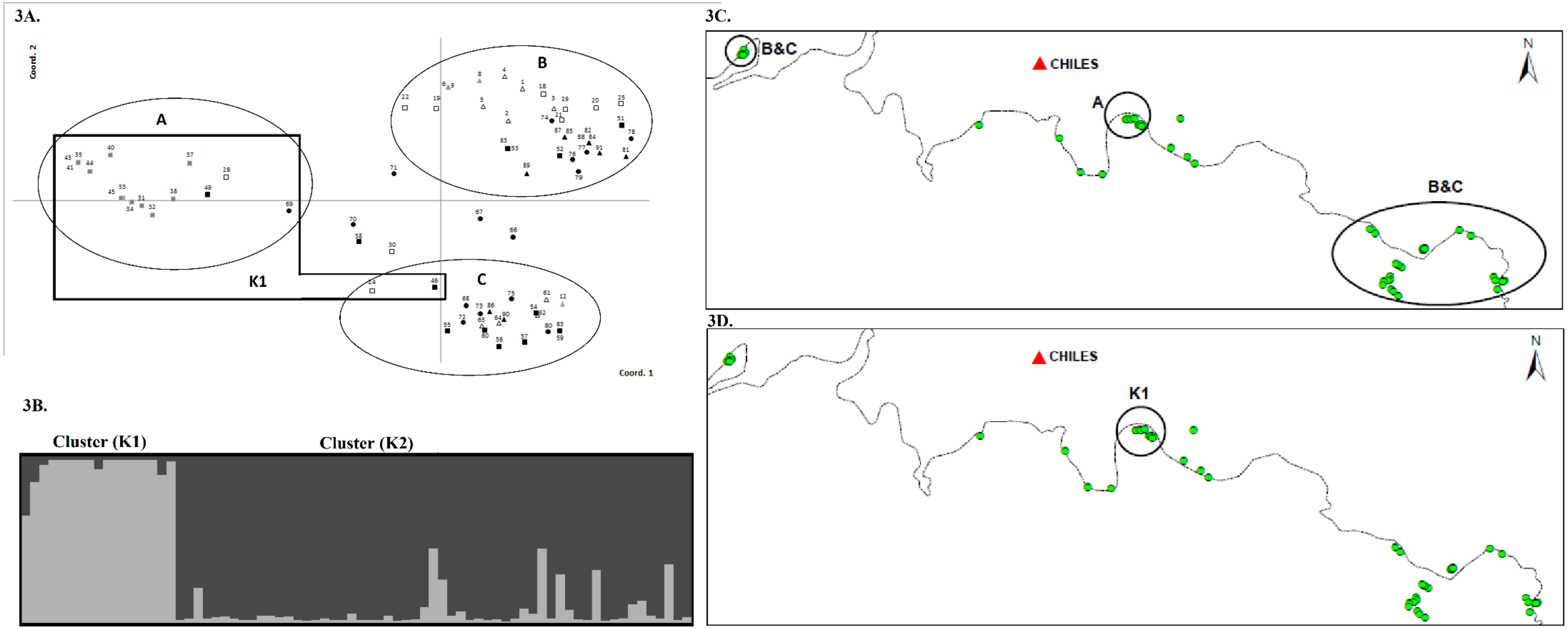

Figure. 3 Mature individuals. **(A)** PCoA and Structure clusters. For PCoA groups A, B and C are clearly defined, the two axis of the PCoA explained the 63.18% of the variance. The triangles represent small groups (three categories differentiated by a gray scale: △ Small Group 1, ▲ Small Group 2 and ▲ Small Group 3), the squares represent big groups (three categories differentiated by a gray scale: □ Big Group 1, ■ Big Group 2 and ■ Big Group3) and the circles ● represent isolated individuals (P). For Structure only cluster K1 is represented, because all the other individuals belong to cluster K2, they are not marked. **(B)** Structure patters, two clusters are clearly defined K1 and K2. **(C)** Spatial location of the groups depicted by the PCoA. **(D)** Spatial location of cluster K1, all the other individuals belong to cluster K2, they are not marked

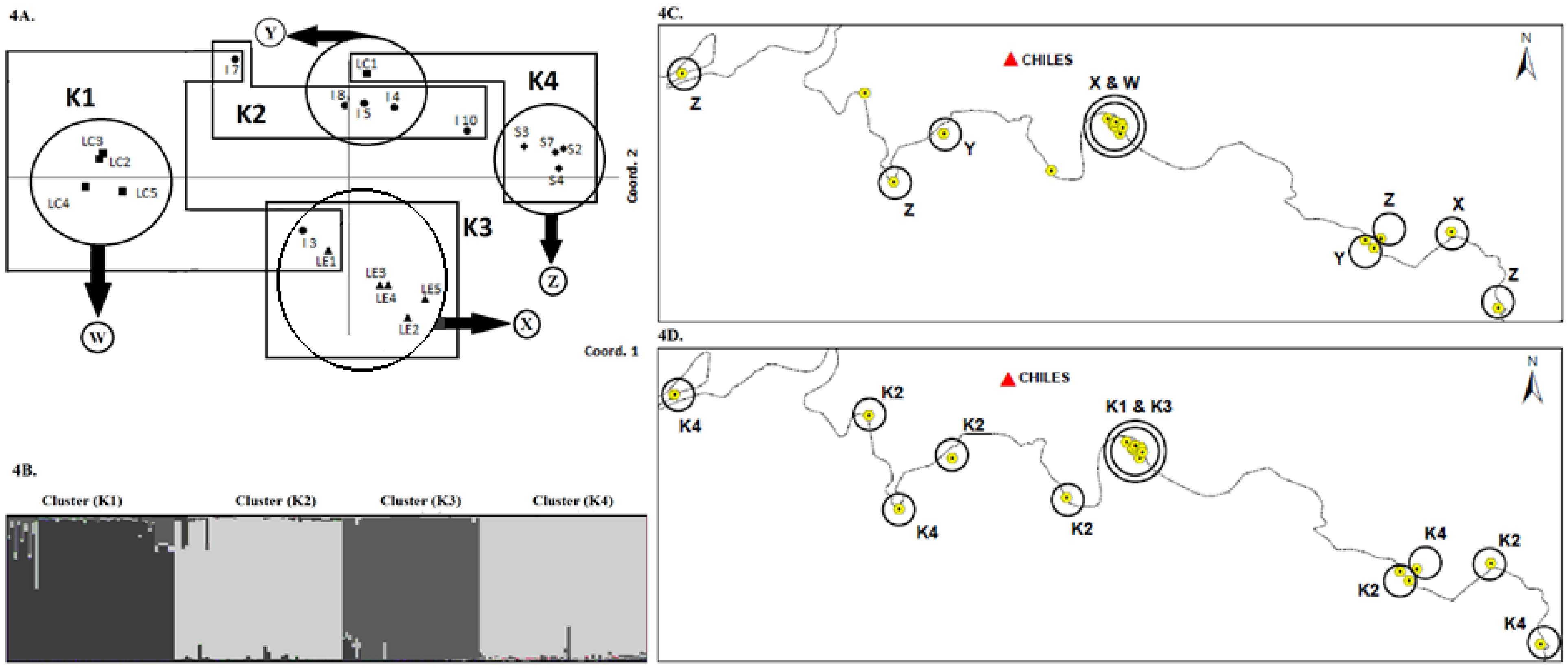

Fig. 4 Infructescences. **(A)** PCoA and Structure clusters. For PCoA groups W, X, Y and Z are clearly defined by circle perimeters, the two axis of the PCoA explained the 62.03% of the variance. The four different categories of infructescences are represent by: circle ● for Isolated, diamond ◆ for small groups, square ■ for center of the big group and triangle ▲ for edge of the big group. For Structure all the four cluster are represented with straight lines perimeters K1, K2, K3 and K4. **(B)** Structure patters, four clusters are clearly defined: K1, K2, K3 and K4. **(C)** Spatial location of the groups depicted by the PCoA. **(D)** Spatial location of the four clusters made by structure.

6. TABLES

Table 1. Genetic structure of six patches and one group of *Puya hamata* derived from five SSRs

Populations	N	A	PA	He	Ho	Fis
SG 1	11	56	0	0,346	0,308	0,213
SG 2	3	14	0	0,122	0,056	0,6
SG 3	11	62	0	0,366	0,523	-0,448
P (Group)	15	82	2	0,47	0,473	-0,339
BG 1	10	51	1	0,367	0,235	0,28
BG 2	12	66	2	0,183	0,111	-0,048
BG 3	12	60	0	0,483	0,326	0,264
Total Mean	11.55	61.67	1	0,36433	0,33115	-0,01747

Genetic Values: (N) = simple size, (A) = number of alleles per population, (P*A*) = private alleles, H*e* = expected heterozygosity, H*o* = observed heterozygosity and F*is* = inbreeding coefficient.

Populations names: (SG) = small group, (P) = isolated individuals and (LG) = big group.

Table 2. Genetic structure of 20 infructescences of *Puya hamata* derived from five SSRs

Infructescences	N	A	PA	He	Ho	Fis
IS 3	10	53	1	0,47344	0,6088	-0,31707
IS 4	10	56	0	0,51996	0,49583	0,19915
IS 5	10	55	3	0,50994	0,55833	-0,29767
IS 7	10	59	0	0,32115	0,48519	-0,58991
IS 8	10	60	1	0,44561	0,45	-0,0104
IS 10	10	58	0	0,47982	0,71667	-0,53571
SS 2	10	56	0	0,4639	0,42963	-0,16547
SS3	10	60	0	0,44035	0,58333	-0,34904
SS4	9	50	0	0,46663	0,4838	-0,15837
SS7	10	57	0	0,49843	0,5463	0,2437
EB 1	10	55	0	0,47365	0,60952	-0,12892
EB 2	10	60	0	0,36404	0,46667	-0,30233
EB 3	10	59	0	0,42686	0,5463	-0,22951
EB 4	10	57	0	0,47504	0,6037	-0,45455
EB 5	4	20	0	0,30833	0,45833	-0,5
CB 1	10	56	0	0,34169	0,41402	-0,28571
CB 2	10	59	0	0,2808	0,45185	-0,71429
CB 3	10	60	0	0,28772	0,4	-0,42105
CB 4	6	31	0	0,32997	0,33333	-0,36364
CB 5	8	42	0	0,37881	0,39583	0,0597
Total Mean	8,2	46,55	0	0,4049525	0,5363075	-0,37678

Genetic Values: (N) = simple size, (A) = number of alleles per population, (P*A*) = private alleles, H*e* = expected heterozygosity, H*o* = observed heterozygosity and F*is* = inbreeding coefficient.

Populations names: (IS) = isolated seeds, (SS) = small group seeds (CB) = seeds of the center of the big group and (EB) = seeds of the edge of the big group.